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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/444,335	11/19/1999	GRIGORI N. ENIKOLOPOV	CSHL99-05	8515

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FISH & NEAVE
1251 AVENUE OF THE AMERICAS
50TH FLOOR
NEW YORK, NY 10020-1105

EXAMINER	
SCHNIZER, RICHARD A	

ART UNIT	PAPER NUMBER
1635	35

DATE MAILED: 01/06/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/444,335	ENIKOLOPOV ET AL.	
	Examiner	Art Unit	
	Richard Schnizer, Ph. D	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1-79* is/are pending in the application.

4a) Of the above claim(s) *25-50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-24 and 51-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/7/03 has been entered.

Claims 1-79 are pending. Claims 25-50 were withdrawn from consideration in Paper No. 13, as being drawn to a non-elected invention. Claims 1-24 and 51-79 are under consideration in this Office Action.

Drawings

The drawings stand objected to for the reasons indicated in the PTO form 948 accompanying Paper No. 13, issued 10/4/00.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 58-65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 58-65 are indefinite because they recite "multipotent stem and progenitor cells of the mouse" without proper antecedent basis. There are two antecedents for "the mouse", the "transgenic mouse" and "a mouse" that provides a fertilized egg. It is unclear to which Applicant refers.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-17, 19-24, 51-71, 78 and 79 are rejected under 35 U.S.C. 102(b) as being anticipated by Zimmerman (1994) as evidenced by Hogan (1986).

Zimmerman teaches transgenic mice comprising a lac-Z transgene under the control of the promoter and second intron enhancer of the rat nestin gene. Beta galactosidase was expressed in neuronal stem cells of the resulting animals, and allowed measurement of these cells. See entire document, especially Abstract; Table I, pages 12, 13, particularly constructs B, C, and F; Fig. 2 on page 15; and page 23, fourth full paragraph. It is noted that beta galactosidase comprises 38 tryptophan residues, and is therefore a fluorescent protein, and cells which comprise it have the property of fluorescence. The mice were made by the method of Hogan, i.e. by coinjection of recombinant expression constructs into the pronuclei of fertilized mouse eggs. See sentence bridging pages 153 and 154; and pages 157-173 of Hogan.

It is noted that a similar rejection was previously withdrawn in Paper No. 28 in view of Applicant's amendments. For example, claim 1, drawn to a transgenic mammal comprising a transgene encoding a marker fluorescent protein was amended to require that "the expression of the gene encoding the marker fluorescent protein is detected using fluorescence." After further consideration it was determined that withdrawal of the rejection was erroneous. One could clearly detect beta galactosidase by either its own fluorescence (e.g. by purifying it from cells and quantitating it by fluorescence spectroscopy), or by detection of fluorescently labeled anti-beta galactosidase antibodies bound to beta galactosidase in situ.

Thus Zimmerman anticipates the claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-24, 51-71, 78, and 79 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Zimmerman (1994) in view of Chiochetti (1997) for the reasons of record in Paper Nos. 13, 17, and 26.

Zimmerman teaches transgenic mice comprising a construct containing a lac Z reporter transgene under the control of the promoter and second intron enhancer of the rat nestin gene. Beta galactosidase was expressed in neuronal stem cells of the

resulting animals, and allowed measurement of these cells. See entire document, especially Abstract; Table I, pages 12, 13, particularly constructs B, C, and F; Fig. 2 on page 15; and page 23, fourth full paragraph.

Zimmerman does not teach a construct comprising green fluorescent protein.

Chiochetti teaches that green fluorescent protein (GFP) is a more powerful and sensitive tool for studying gene expression in transgenic animals than is beta galactosidase. See entire document, especially page 202, column 1, lines 5-7. Chiochetti also teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined. See page 201, column 1, lines 11-14.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Zimmerman by substituting green fluorescent protein for beta galactosidase, and to study gene expression in neuronal stem cells in living animals and their organs and tissues. One would have been motivated to do so because Chiochetti teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined through the use of GFP. See page 201, column 1, lines 5-14.

Thus the invention as a whole was *prima facie* obvious.

Claims 72-77 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zimmerman (1994) and Chiochetti (1997) as applied to claims 1-24, 51-71, 78, and 79 above, and further in view of Yeh et al (Proc. Nat. Acad. Sci. USA 92:7306-7040, 7/1995), Lois et al (Science 264(5162):1145-1148, 5/1994), and Reynolds et al (Science 255(5052):1707-1710, 3/1992).

Zimmerman who teaches a method of making a transgenic animal comprising a lac-Z transgene under control of the promoter and second intron enhancer of the rat nestin gene, and the detection of neuronal stem cells in these animals for the purpose of analyzing gene expression in these cells. See entire document, especially abstract; page 11, last sentence of paragraph bridging columns 1 and 2; sentence bridging pages 11 and 12; Table 1, pages 12 and 13, particularly constructs B, C, and F; Fig. 2 on page 15; and page 23, fourth full paragraph. Zimmerman does not teach a method of measuring multipotent stem and progenitor cells wherein the measurement step is carried out in a live animal.

Chiochetti teaches that green fluorescent protein (GFP) is a more powerful and sensitive tool for studying gene expression in transgenic animals than is beta galactosidase. See entire document, especially page 202, column 1, lines 5-7. Chiochetti also teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined. See page 201, column 1, lines 11-14.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Zimmerman by substituting green fluorescent protein for beta galactosidase, and to study gene expression in neuronal stem cells in living animals and their organs and tissues. One would have been motivated to do so because Chiochetti teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined through the use of GFP. See page 201, column 1, lines 11-14. Furthermore, Yeh teaches that GFP can be monitored in intact, living embryos, and can be used for a variety of purposes including measurement of dynamic changes in gene expression in living tissue; lineage analysis;

and monitoring cell migrations and changes in cell shape. See abstract; the last two sentences of the first full paragraph of column 2 on page 7036; page 7040, column 1, lines 5-9, and first two sentences of paragraph bridging columns 1 and 2 on page 7040. In addition, Lois et al teach the study of migration of neuronal precursors in adult mammalian brain, and Reynolds teaches that adult neuronal stem cells express nestin. See abstracts. Given the teachings of the prior art as discussed above, one of ordinary skill in the art would have been motivated to use a transgenic animal comprising a GFP sequence under the control of nestin regulatory sequences to follow neuronal precursor migration in living animals. In summary, the prior art provides explicit statements that GFP is superior to beta galactosidase for use in transgenic animals, and that it should be used to study cells of transgenic animals *in vivo*. The prior art also teaches that nestin transcription control sequences can be used to study neuronal precursor cells, and provides motivation to examine the transcriptional activities of these cells as well as their migration and morphology. Armed with this information, one of ordinary skill in the art would clearly be motivated to use nestin/GFP constructs in transgenic animals for the purpose of studying neuronal precursor populations.

Thus the invention as a whole was *prima facie* obvious.

Response to Arguments

Applicant's arguments, filed 10/7/03 have been fully considered but they are not persuasive.

An Advisory Action was issued 4/29/03 which stated that Applicant's previous arguments, filed 4/7/03 were unpersuasive. Applicant had argued that the cited art failed to render obvious the invention because it failed to teach or suggest whole body imaging. This was found to be unpersuasive because the none of the claims required whole body imaging.

Applicant's current argument is set forth at pages 19 and 20 of the response. Applicant asserts at page 21, first paragraph of the current response that, in their response to a final rejection issued (10/7/02) they argued that the invention was distinguishable over the cited art because it "unexpectedly allows real time, whole body imaging of a mammal." A review of the previous response, filed 4/7/03 shows that Applicant did not cast the issue in terms of unexpected results. Applicant indicated only that the cited art failed to render obvious the invention because it failed to teach or suggest whole body imaging. As such the resulting Advisory Action indicated that the arguments were unpersuasive because Applicant argued limitations that were not in the claims.

Applicant asserts at pages 19 and 20 of the instant response that evidence relied upon relating to unexpected results or advantages need not be disclosed in the specification. This is true, but it is not at issue. What is at issue is whether or not the Declaration of Dr. Hoffman, filed 8/6/02 provides evidence of unexpected results that overcomes the obviousness rejections. Dr. Hoffman examined transgenic mice of the instant invention and declared that he "was surprised to detect such strong, well defined fluorescence in a live, young adult transgenic animal that had integrated into its genome, GFP, as a reporter of nestin gene expression. I was also surprised by the presence of GFP under control of regulatory elements of the nestin gene in the

pancreas and testes of these transgenic mice and by the intensity and definition of the fluorescence observed in these organs." Dr Hoffmann also states that he "would not have expected that a transgenic mouse that had had integrated into its genomic DNA, the GFP gene under the control of regulatory elements of the nestin gene, and that was produced by introducing into a fertilized egg, DNA that included GFP under the control of regulatory elements of the nestin gene, would exhibit fluorescence intensity and fluorescence definition that we observed in our work with Dr. Enikolopov's mice." These statements represent an opinion of one of skill in the art, but do not represent evidence that any difference between the results presented by Applicant and those in the prior art are significant. MPEP 716.02(b) discusses the burden on Applicant in overcoming an obviousness rejection by presentation of evidence of unexpected results. "The evidence relied up [sic] should establish "that the differences in results are in fact unexpected and unobvious and of both statistical and practical significance." Ex parte Gelles, 22 USPQ2d 1318, 1319 (Bd. Pat. App. & Inter. 1992). In this case, it has not been established that the obtained results are statistically or practically different from those of the prior art. It was known in the prior art that the nestin promoter was an effective promoter for expression of beta galactosidase in transgenic animals (Zimmerman, 1994). It is clear that there is motivation to substitute GFP for beta galactosidase (See, Chiochetti 1997). Absent evidence to the contrary, one of ordinary skill in the art could reasonably expect a similar level of GFP expression as was obtained for beta galactosidase. Applicant has provided no evidence that GFP would be expressed at any unexpectedly greater level than was beta galactosidase, i.e. Applicant has presented no evidence that the level of GFP expression obtained was unexpected. Because the fluorescence intensity is directly related to the quantity of

GFP produced, there is no reason of record to expect any less fluorescence than that which was observed by Applicant. For these reasons the rejection is maintained.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 703-306-5441 until 1/13/04, and thereafter will be 571-272-0762. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Andrew Wang, can be reached at 703-306-3217 before 2/22/04, and at 571-272-0811 after 2/22/04. The official central fax number is 703-872-9306 until further notice. Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Trina Turner whose telephone number is 703-305-3413 prior to 1/14/04, and thereafter will be 571-272-0564.

DAVE T. NGUYEN
PRIMARY EXAMINER

Richard Schnizer, Ph.D.